# LIGHT STIMULATED RNA SYNTHESIS IN ZEA LEAVES

by

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In the higher plants the formation of chloroplast can be separated into three phases:

These are: 1. multiplication or self-duplication,
2. differentiation; formation of structure and function;
3. compartmentation, photosynthetical activity.

Recently our knowledge about these phases has been remarkably enlarged, still there are some unexplained questions in relation to genetical problems.

Differentation is the longest and most spectacular phase in the development of the chloroplasts (Wettstein 1958, Mühlethaler and Frey-Wyssling 1959.).

In higher plants the differentiation of chloroplast can be separated into well definable stages. There are proplastid → etioplast → chloroplast. The proplastid → etioplast transformation is carried out in the dark (Wehrmeyer 1965) while the formation of chloroplast from etioplast requires light in the *Angiospermae* (Gunning and Jagoe 1967).

In addition to the well known structural and functional ones, light causes significant changes also in genetical regulation too; *de novo* synthesis of nucleic acids and proteins can be observed (Smillie and Krotkov 1960, Aoki and Hase 1964, Brawerman et al. 1962). This effect could not been always observed by others as to be seen from the literature (Feierabend and Pirson 1966, Duranton 1966.).

It is clear that the light produces its effects through some photoreceptors (for instance protochlorophyllide-holochrom, chlorophyll-protein complex, pytochrome and the receptors of blue light: flavins and carotenoids) which probably localised in the chloroplasts.

It is well-known that the chloroplast has a genetical system of its own and it contains a machinery of protein synthesis (Smillie and Scott 1969, Sissakian et al. 1965). The results of investigations were carried out in this field show that light stimulates the biosynthetical processes which are coded by chloroplast DNA (Parthier 1962).

Starting from these facts in our laboratory we studied the light stimulated synthesis of RNA in normal and chloroplast mutant leaves.

In this connection, there are still a number of questions to be explained. For instance: 1. what is the rate of light induction of RNA; 2. is there any direct effect of light in RNA synthesis; 3. what is the role of the photoreceptor or photoreceptors; 4. is there a "*de novo*" synthesis of messenger RNA?

In the work discussed here we determined the effect of light stimulation in nucleic acid synthesis.

## Material and methods

*a) Plant material*

The test material consisted of normal *Zea mays L.* individuals. The plant were grown in the dark and in light for 10 days at 25 °C and the rough weight as well as nucleic-acid content of leaves were determined by using samples taken every day.

In the other experiments the plant were grown the dark for 6 – 7 days, and the chloroplast development was then stimulated by illumination at 1000 lux light intensity.

*b) Chloroplast isolation*

A method for the isolation of intact chloroplasts was described in detail by Leech (1964). 1 – 2 g of leaf laminae were homogenized in a mortal containing 3 – 4 fold chilled isolation medium (0.4 M sucrose in 0.15 M phosphate buffer, pH 7.3). The resultant brei was filtered through muslin and centrifuged at 600 xg for 2 minute. The supernatant was centrifuged once more at 1000 xg for 12 minute and the resuspended pellets were layered onto a discontinuous gradient composed of two layer of 60% and 25% glycerol (v/v) in buffered sucrose. After a centrifugation at 1000 xg for 20 minute the chloroplast layer was removed with an injector and was used for determination of nucleic-acids.

*c) Isolation of DNA and RNA from leaves and isolated chloroplasts*

Nucleic-acid isolation was done by the method of Nechayeva (1966): the leaves or chloroplast fraction were homogenized with 5% $HClO_4$, at o °C. After centrifuging the homogenate at 10 000 xg, the precipitate was repeatedly washed wit 96% alcohol, a 1:1 ratio mix-

ture of alcohol and ether, and with ether. For nucleic-acid hydrolysis, the precipitate was kept in 0.5 n KOH solution at 37 °C for 18 hours, then the hydrolysate was cold centrifuged. In order to separate the DNA and RNA fractions, the supernatant was neutralized with $HClO_4$ solution then, after 20 min rest, acidified to a 5% final concentration by adding another quantity of $HClO_4$ solution. Following a short rest period and centrifuging (20 000 xg), the supernatant characterized RNA while DNA appeared in the precipitate. DNA was hydrolyzed by boiling with 5% $HClO_4$ at 90 C°.

The density of the RNA and DNA fractions was measured after repeated hydrolysis at 90 °C and 260 m by means of a spectrophotometer. The nucleic-acid quantity was determined from the optical density of the fractions thus obtained, by using a calibration curve.

*d) Phenol extraction of RNA from whole leaves*

Native RNA was extracted from whole leaves with phenol, using Method A of L o e n i n g and I n g l e (1964). The fresh leaves were homogenized at 0 °C in a mortar in 10 mM tris pH 7.4, containing 1 per cent sodium laurylsulphate and 12 mg/ml of bentonite plus an equal volume of phenol containing 0.1 per cent 8-hydroxyquinoline and 10 per cent m-cresol. After centrifugation the lower phenol layer was removed to leave the interphase and aqueous layer to which sodium chloride (final concentration, 0.5 molar) and an equal volume of phenol mixture were added. After mixing and centrifuging, the top aqueous layer was removed and extracted once more with phenol. Two volumes of ethanol were added to precipitate the nucleic acids from the final aqueous layer. The precipitate was dissolved in 0.15 molar sodium acetate, pH 6.0, containing 0.5 per cent sodium laurylsulphate, and reprecipitated with two volumes of ethanol. After centrifugation the pellet was washed with cold 80 per cent ethanol, partially dried and dissolved in the electrophoresis buffer.

The RNA fractionation was achieved by polyacrylamide gel electrophoresis (L o e n i n g 1967). Gels were prepared containing 2.5 per cent acrylamide and 0.12 per cent bisacrylamide. The electrophoresis buffer contained 0.4 M tris, 0.02 M sodium acetate, 2 mM EDTA and 5% (w/v) of sucrose; acetic acid was used to adjust the pH to 7.8.

*e) Procedure for electrophoresis*

Electrophoresis was carried out at about 5 °C in a refrigerator and 5 mA/gel was applied. Suitable separations were obtained in 2 hours. After the run the gels were gently blown out of the tubes and cut into pieces after freezing for measuring their $^{32}P$ activity.

*f) Determination of radioactivity*

For studying RNA synthesis by $^{32}P$-labelling the etiolated Zea leaves were placed into an aqueous solution of $^{32}P$-orthophosphate for 10 hours. After extraction and separation of leaf RNA by polyacrylamide gel electrophoresis we cut the gels into pieces and measured their activity on the basis of Cherenkoff-effect in a liquid scintillation spectrometer.

## Results

Figures 1. and 2. show the change of fresh weight and nucleic-acid content at different ages of normal leaves.

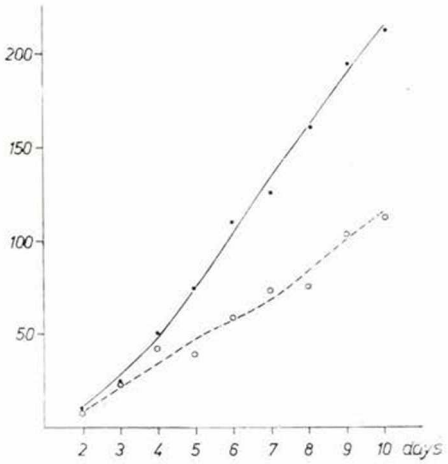


Fig. 1 The change of fresch weight in normal Zea leaves of different ages. – . – in the light; – – – o – – – in the dark;

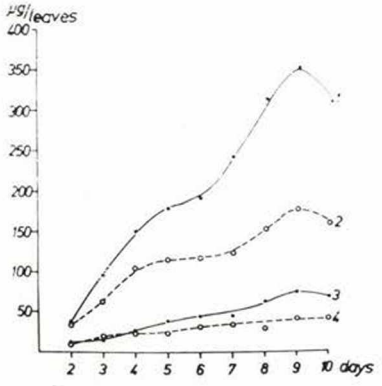


Fig. 2 The change nucleic-acid content determined in normal leaves of different ages.

1. RNA content in the light;
2. RNA content in the dark;
3. DNA content in the light;
4. DNA content in the dark;

We can see that the fresh weight gradually increases with time. The light-effect can be observed after 4 days.

As regards the nucleic-acids, the RNA content per leaf is always higher in the light after 4 days, but the profiles of the dark and light curve are similar. The variation in RNA content does not closly follow the gain in fresh weight. RNA content has two definite maxima.

Table I shows the RNA/DNA ratios.

*Table I.*

**RNA/DNA rations in illuminated and dark grown leaves**

| Age of seedlings (days) | RNA/DNA ratio | |
|---|---|---|
| | light | dark |
| 2 | 2.92 | 3.10 |
| 3 | 5.50 | 2.95 |
| 4 | 5.28 | 4.42 |
| 5 | 4.62 | 4.20 |
| 6 | 4.33 | 3.62 |
| 7 | 5.45 | 3.68 |
| 8 | 4.92 | 4.12 |
| 9 | 4.96 | 3.77 |
| 10 | 4.42 | 4.02 |

It is easy to see well that the RNA/DNA ratios are at any time higher in the light. Light firstly stimulates RNA synthesis because the DNA content is nearly constant as to be seen in Fig. 2.

In order to obtain more detailed information about the light-induction process the correlation between RNA synthesis and chloroplast differentation was investigated. For this purpose six days old etiolated seedling were illuminated for 5, 10, 30, 60 minutes, 6 and 24 hours. Then were kept the seedlings in dark and the nucleic acid content of the leaves was measured with 24 hours beginning after the light treatment.

The results of nucleic-acid accumulation are presented in Fig. 3.

The amount of leaf RNA significantly increases in the course of greening. The RNA/DNA ratios also show this light stimulated process.

When we measured the RNA content in isolated chloroplasts (Table II) we found the same tendency as in the whole leaves.

*Table II.*

**Effect of preillumination on the nucleic acid content of isolated chloroplasts of Zea leaves**

| Preillumination (minute) | RNA /g | DNA /g | RNA/DNA |
|---|---|---|---|
| 0 | 279.0 | 151.4 | 1.84 |
| 5 | 91.0 | 35.8 | 2.54 |
| 60 | 126.0 | 50.4 | 2.52 |
| 6.60 | 261.0 | 103.7 | 2.53 |
| 24.60 | 336.0 | 107.0 | 3.10 |

The question arose wether light-stimulated RNA synthesis was localized in the chloroplasts or in an other place of the cell.

We have two other experimental data which indicate the localization of light stimulated RNA synthesis. The experiments were conducted with chloroplast-mutant plants. Table III shows RNA synthesis in illuminated leaves of normal, lycopenic and ξ-carotenic mutant of *Zea* seedlings.

*Table III.*

**The effect of illumination on the RNA synthesis of normal and mutant leaves (light/dark)**

| Material | Illumination (hours) | | |
|---|---|---|---|
| | 3 | 6 | 24 |
| normal | 3.3 | 4.7 | 4.6 |
| lycopenic | 1.0 | 1.0 | 1.2 |
| ξ-carotenic | 1.0 | 1.2 | 1.2 |

From the data it appears that in mutant plants the light/dark ratios of RNA labelled with $P^{32}$ are nearly 1.0 in the mutant. This means that light-stimulation could not be observed in them.

The other proof is coming from the experiments with variegated leaves of *Tradescantia albiflora* cv. *aureovittata*. Table IV shows the variation of RNA and DNA content in green and in white tissues of leaves.

It is to be seen from the Table that the RNA/DNA ratios are lower in white tissue containing degenerate plastids.

The next step of our studies was to follow the synthesis of different RNA species by $P^{32}$-labelling. Etiolated *Zea* leaves were placed into an aqueous solution of $P^{32}$-orthophosphate for 10 hours. After

*Table IV.*

**Content of DNA and RNA in white and green tissues of variegated Tradescantia leaves**

| Variant | No | DNA µg/ml | RNA µg/ml | RNA/DNA |
|---|---|---|---|---|
| White tissue | 1. | 5.8 | 8.2 | 1.4 |
| | 2. | 4.5 | 5.0 | 1.1 $\bar{x}$ = 1.3 |
| | 3. | 2.8 | 4.4 | 1.5 |
| Green tissue | 1. | 3.0 | 7.5 | 2.5 |
| | 2. | 8.2 | 18.4 | 2.2 $\bar{x}$ = 2.7 |
| | 3. | 2.6 | 8.0 | 3.3 |

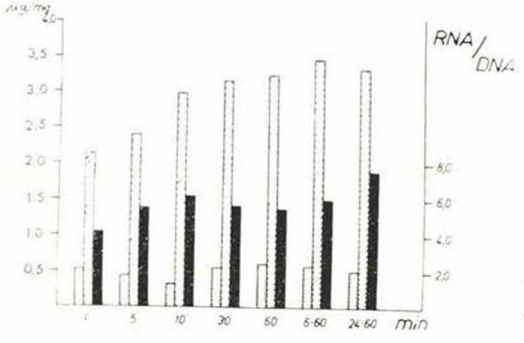


Fig. 3 The change of nucleic acid content and RNA/DNA ratios in leaves of Zea seedlings illuminated at different time. Horizontically marked = DNA, vertically marked = RNA, dark shaded = RNA/DNA

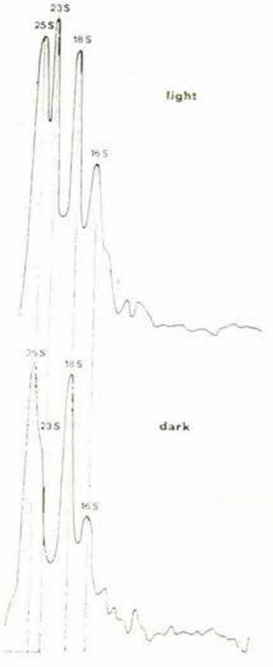


Fig. 4 Profiles of radioactivity ($P^{32}$) in polyacrylamide gels of RNA from illuminated and dark grown leaves.

that, they were illuminated for 12 hours with an intensity of 1000 lux. Native RNA was extracted from the whole leaves with phenol and the RNA fractions were separated by polyacrylamide gel electrophoresis.

Fig. 4. shows the radioactive profile of gels.

It can be see that the labelling of chloroplast ribosomal RNA, namely the 23S and 16S fractions increased in the light. Unfortunately we could not exactly observe the labelling of soluble RNA fractions in experiments.

## Discussion

Our experimental data (Fig. 1 – 2) reveal that the variation of the nucleic-acid content does not follow the increase in weight of the leaves in the course of growing. Similar results could be observed in the experiments conducted by Rhodes and Yemm (1966) on barley seedling and by Brady an coworkers (1971) on wheat plants. In wheat seedlings grown in the light the capacity of the leaves to incorporate of orotic acid into ribosomal RNA decreases rapidly and the extent of the decrease in much greater chloroplast ribosomal RNA as compared with cytoplasmic RNA (Patterson and Smillie 1971).

The amount of leaf RNA significantly increases during the greening of etioplast. In *Euglena* measurement of RNA isolated from chloroplast fractions and from whole cells using dark grown and greened cultures reveals a synthesis of both cytoplasmic and chloroplastic RNA during chloroplast development. In *Phaseolus vulgaris* Gyldenholm (1968) observed a 50 per cent increase in chloroplast associated RNA during 45 hours greening of dark grown primary leaves. The increase amounted to approximately the half ot the total increase of RNA. Experiments with leaves of bean seedlings (Anderson 1971) showed that both cytoplasmic and chloroplastic ribosomes could be labelled throughout the period of chloroplast development. The degree of ribosomal RNA labelling was slightly higher in the chloroplast than in the cytoplasm during the period of grana formation. The measurement of RNA polymerase activity show that this enzime is forming and decaying in etiolated maize proplastids in darkness but that the rate of formation is increased by light (Bogorad 1967).

The changes in plastid RNA metabolism and RNA polymerase that occur during light-induced plastic development may have a role in the control of plastid development – or at least in the light-stimulated production of certain proteins.

Our experiments with chloroplast-mutant plants, first of all with cytoplasmic mutants show that the lightinduced RNA synthesis is mainly located in the chloroplast.

Ingle (1968) observed that the chloroplast was synthesized in dark-grown radish cotyledons at a rate of about one third of that in

the light. The synthesis, however, continous for a longer time in the dark, and the percentage of chloroplast RNA can approach that in lightgrown tissue. These results are similar to ours and indicate that continuous light is not essential for the production of ribosomal RNA in plastids. This is one of the characteristic of phytochrome systems. It is well known that phytochrome is a redfar red photoreversible pig-ment system. It acts as photoreceptor in these light-regulated processes. Several possible mechanisms of action include: *a)* control of membrane permeability (Hendricks and Borthwick 1967); *b)* differential gene activation (Schopfer 1971); c*)* phytochrome acting as an enzym (Mohr 1962, Siegelman and Hendricks 1964). It seems that the studies are consistent with the theory of differential gene activation.

As we know the photomorphogenic changes which occur following illumination of etiolated leaves, expansion and greening are accompained by an increased capacity of RNA synthesis. These observations raised several questions. First, what kind of RNA is synthesized, and second, what RNA fractions are necessary for the photomorphogenic changes? The data arising from polyacrylamide gel analysis experiments clearly show the light-stimulated accumulation of ribosomal RNA, namely 23S and 16S species in chloroplast.

## Summary

The data reported shown that illumination stimulates RNA synthesis in the course of leaf growth and greening. Analysis of purified chloroplasts and experiments with nuclear and cytoplasmic mutants and $P^{32}$ labelling of nucleic-acids led to the conclusion that light stimulation of RNA synthesis was localized in chloroplast.

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